REPORT of the SHORT TERM SCIENTIFIC MISSION, COST FA1208-ID n°

Title: Validating interactors for the Blumeria Effector Candidate BEC1054

Start date: 2014-08-04 **End date:** 2014-11-04

Applicant: Ms Helen Pennington, Imperial College London, (UK)

Host: Ralph Panstruga, RWTH Aachen University, Aachen (DE)

Background:

Powdery mildews are some of the most frequently encountered plant pathogenic fungi. They infect almost 10,000 plant species including ornamental plants, tree fruits, grapes, hops and grains, and have a significant detrimental economic impact (Braun *et al.*, 2006, Dean *et al.*, 2012). One plant affected is barley (*Hordeum vulgare*), which is mainly used to produce animal feed and for malting (Gale & Devos, 1998).

Barley powdery mildew, *Blumeria graminis* formae speciales *hordei*, is the most frequently used powdery mildew for the investigation of cellular and molecular level host-pathogen interactions. It produces proteins, called effectors, which help it to infect barley (Both & Spanu, 2004, Bindschedler *et al.*, 2011). These effectors include Blumeria Effector Candidate 1054 (BEC1054), which has been found to contribute to virulence and to significantly affect infection. Analyses of BEC1054's structure and function have indicated that BEC1054 has originated from an extracellular RNAse, similar to the RNAse T1 family, but that it does not possess the catalytic sites required for RNAse activity (Pedersen *et al.*, 2012, Pliego *et al.*, 2013).

Putative protein interactors of BEC1054 were identified through *in vitro* chromatography, and 247 proteins were found which associate with BEC1054. A number of these target proteins have been tested in yeast, through the use of a yeast-two-hybrid assay. A Glutathione-S-transferase (GST), a Pathogenesis-Related protein 5 (PR5), a PR10, and elongation factor 1 gamma (eEF1G) all showed evidence of interaction with BEC1054.

Objectives/ Purpose of the visit:

AIM: To further validate whether BEC1054 interacts with proteins including GST, PR5, PR10 and eEF1G in the host plant barley.

OBJECTIVE: To use Bimolecular Fluorescence Complementation (BiFC) to analyse the interactions of BEC1054 with its putative interactors.

Description of the work:

In BiFC analysis, two proteins of interest (i.e. BEC1054 and GST) are fused to two fragments of a fluorescent reporter protein (Figure 1). The interaction of the two proteins of interest brings the two fluorescent fragments together. This allows the fluorescent reporter to reform, causing it to emit a fluorescence signal. Confocal microscopy can be used to both detect and locate the signal; and the intensity of the signal indicates the strength of the interaction, with low levels indicating interaction within a complex, and high levels indicating direct interaction.

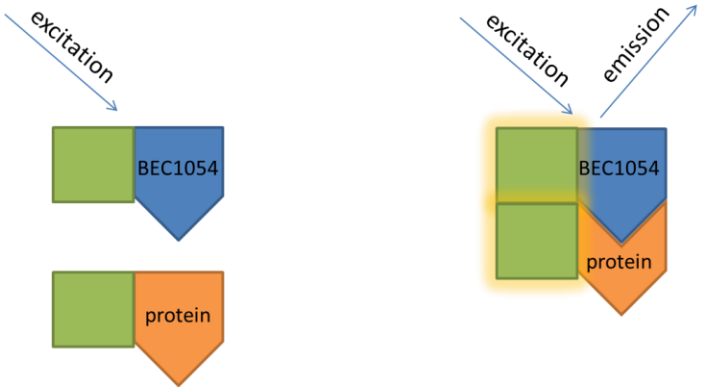

Figure 1: Basis of Bimolecular Fluorescence Complementation (BiFC). Two proteins of interest (i.e. BEC1054 and GST) are expressed fused to two fragments of a fluorescent reporter protein. The interaction of the two proteins of interest brings the two fluorescent fragments together. This allows the fluorescent reporter to reform, causing it to emit a fluorescence signal following excitation.

The bait and prey proteins were cloned into entry vectors (pCR8 or pDONR201), and then recombined into expression vectors. These were used to coat gold microcarriers, which were then used to bombard eight-day-old leaves from *H. vulgare* c.v. Golden Promise, grown under long day conditions. Three days post bombardment; leaves were imaged through confocal microscopy to determine whether an interaction had taken place.

Main results:

A series of nine proteins were assayed through BiFC to determine whether they interacted with BEC1054. Three of these, GST, PR5 and eEF1a (Figure 2), were found to interact. The interactions with PR5 and GST support those of our Y2H work, providing further evidence that BEC1054 does interact with these proteins. The additional interaction with eEF1a, and the absence of an identifiable interaction with eEF1g indicate the need to investigate protein-protein interactions using more than one method. Furthermore, the absence of an interaction in both Y2H and BiFC studies may be due to miss-folding of the fused proteins; or due to the absence of interaction partners. The interaction with eEF1a identified in barley, but not in yeast, may be due to differences present between the yeast and barley ribosomes.

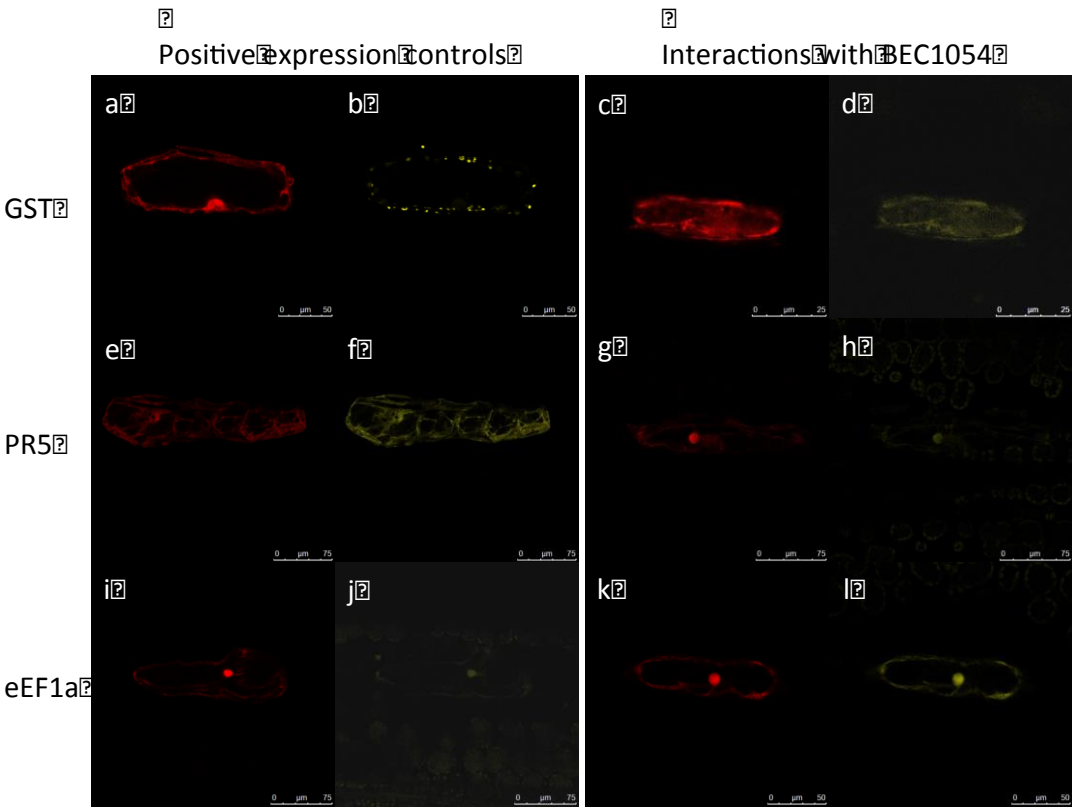

Figure 2: BEC1054 interacts with Glutathione-S-Transferase (GST), Pathogenesis Response Protein 5 (PR5) and Eukaryotic Elongation factor 1 Alpha (eEF1a). Red images indicate detected dsRED signal, which was used as an indication that cells had been transformed. Yellow images indicate detected YFP signal. The 'positive expression controls' (a, b, e, f, i, and j) indicate that the three proteins GST, PR5 and eEF1a are expressed when fused with a monomeric YFP molecule. The 'Interactions with BEC1054' (c, d, g, h, k and l) indicate the YFP signal detected upon the interaction of BEC1054 with the prey proteins.

Future collaboration with the host institution (if applicable):

The BiFC project is currently ongoing, with the remaining interactors being assayed to determine whether they interact with BEC1054 in barley.

The host and home laboratories are now continuing collaboration on a BBSRC/EU funded project (ERA-CAPS programme; Title: Functional characterisation and validation of non-host components in *Triticeae* species for durable resistance against fungal diseases). Two researchers (PDRA and PhD) are scheduled to work on this over the next 4 years.

Projected publications/articles related to or resulting from the STSM:

The *B. graminis* RNAse like effector BEC1054 interacts with multiple proteins in the host plant, barley [*In preparation*].

References

Bindschedler, L. V., McGuffin, L. J., Burgis, T. A., Spanu, P. D. and Cramer, R. (2011) Proteogenomics and in silico structural and functional annotation of the barley powdery mildew Blumeria graminis f. sp hordei. *Methods,* **54,** 432-441.

Both, M. and Spanu, P. D. (2004) Blumeria graminis f. sp. hordei, an obligate pathogen of barley. *Plant-pathogen interactions***,** 202-218.

Braun, U., Takamatsu, S., Heluta, V., Limkaisang, S., Divarangkoon, R., Cook, R.*, et al.* (2006) Phylogeny and taxonomy of powdery mildew fungi of Erysiphe sect. Uncinula on Carpinus species. *Mycological Progress,* **5**.

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D.*, et al.* (2012) The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology,* **13**.

Gale, M. D. and Devos, K. M. (1998) Comparative genetics in the grasses. *Proceedings of the National Academy of Sciences of the United States of America,* **95,** 1971-1974.

Mazel, A. and Levine, A. (2001) Induction of cell death in Arabidopsis by superoxide in combination with salicylic acid or with protein synthesis inhibitors. *Free Radical Biology and Medicine,* **30**.

Pedersen, C., van Themaat, E. V. L., McGuffin, L. J., Abbott, J. C., Burgis, T. A., Barton, G.*, et al.* (2012) Structure and evolution of barley powdery mildew effector candidates. *Bmc Genomics,* **13**.

Pliego, C., Nowara, D., Bonciani, G., Gheorghe, D. M., Xu, R., Surana, P.*, et al.* (2013) Host-Induced Gene Silencing in Barley Powdery Mildew Reveals a Class of Ribonuclease-Like Effectors. *Molecular Plant-Microbe Interactions,* **26,** 633-642.